Acetolysis of 1,6-Anhydro-β-D-gulopyranose.—The reaction of 1 g. of the anhydride II with 50 ml. of acetic anhydride and 0.5 ml. of concentrated sulfuric acid at 20° led to a constant rotation of $[\alpha]^{20}D$ +15.3° (calcd. as D-gulose pentaacetate) at the end of 16 hours, with no further change during the next 6 hours. The product, isolated in the usual manner, was a sirup that could not be induced to crystallize.

The acetolysis of a second 1-g. portion of anhydride, in 25 ml. of acetic anhydride and 0.5 ml. of sulfuric acid, was carried out for 4 days at −5° in the hope that at the lower temperature a larger amount of the crystalline α-anomer might be formed and its rearrangement to the unknown β-anomer be retarded. Although no rotation was taken to confirm those expectations, the final result was the isolation of about 0.2 g. of the known α-D-gulose pentaacetate.[9] Recrystallized from ether–pentane, the product—large acicular prisms—melted at 106–107° and showed no depression of m.p. when mixed with an authentic sample furnished through the courtesy of Drs. Frush and Isbell. The rotation, $[\alpha]^{20}D$ +86.6° in chloroform (*c* 0.24), was in good agreement with the value +86.2° reported by those authors.

(9) H. L. Frush and H. S. Isbell, *J. Research Natl. Bur. Standards*, **34**, 111 (1945).

Acknowledgment.—The authors wish to thank Dr. Robert K. Ness, Dr. James W. Pratt, Mr. John T. Sipes and Mr. Edward W. Tracy for preparing and supplying the D-gulose and the methyl α-D-gulopyranoside tetraacetate; and Dr. William C. Alford and his associates of this Institute's microanalytical laboratory for carrying out the microanalyses.

BETHESDA 14, MARYLAND

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, IOWA STATE COLLEGE]

Isolation and Identification of Planteose from Tobacco Seeds[1]

BY DEXTER FRENCH

RECEIVED SEPTEMBER 27, 1954

A crystalline trisaccharide prepared from tobacco seeds is found to be identical with planteose, *O*-α-D-galactopyranosyl (1 → 6)-*O*-β-D-fructofuranosyl-(2 → 1)-α-D-glucopyranoside.

Recently Wada and Yamazaki reported[2] the existence of a trisaccharide isomeric with raffinose in seeds of tobacco, *Nicotiana tabacum*. This trisaccharide differed from raffinose in its paper chromatographic behavior and in its cleavage by mild acid hydrolysis to D-glucose and a ketose disaccharide of D-fructose and D-galactose. These properties are similar to those recorded for planteose,[3,4] a trisaccharide from seeds of various *Plantago* species.

In order to make a more detailed examination of the trisaccharide, it was prepared on a macroscopic scale and its properties were compared with those of planteose (Table I). The close similarities of the characteristic properties of planteose from *Plantago* seeds and tobacco seed trisaccharide, together with the identical appearance of the X-ray diffraction powder patterns, point to the identity of the trisaccharides from these two rather distantly related plants.

Experimental

Four hundred grams of tobacco seeds, variety Yellow Mammoth,[5] was ground overnight in a ball mill to remove the seed coat and partially break down the seed structure. The seeds tended to pack in the mill because of the high apparent oil content and after brief extraction with diethyl ether the seeds were returned to the ball mill for a second overnight grinding.

The entire batch of ground seeds was extracted in a large Soxhlet apparatus, first for 24 hours with diethyl ether, then for 72 hours with 99% methanol. The methanolic extract was concentrated to about 200 ml. by evaporation in a warm air stream and treated with 200 ml. of petroleum ether and 200 ml. of acetone. The upper phase was discarded and the lower phase was again treated with 200 ml. of acetone and 300 ml. of petroleum ether. All was discarded except a gummy precipitate which had appeared during the concentration and extraction of the methanol extract.

The gummy precipitate was washed briefly with methanol and acetone and dissolved in 125 ml. of water. Paper chromatography[6] showed that it contained sucrose and trisaccharide in a ratio of 3 or 4 to 1, and measurement of the optical rotation indicated the presence of about 2.3 g. of material, assuming that it consisted only of sucrose and planteose with an average specific rotation for the mixture of +81.

Charcoal Separation of Tobacco Seed Sugars.—The entire sugar solution was diluted to 1 liter and placed upon a 40-g. charcoal column.[7] One liter eluates with dilute ethanol were examined for total optical rotation and for qualitative composition by paper chromatography (Table II).

Crystallization and Identification of Tobacco Seed Sucrose.—The 2% ethanol eluate from the charcoal column was evaporated to a sirup and purified by large scale paper chromatography. Crystals which formed from ethanol–acetic acid were removed by filtration, washed briefly with 85% ethanol and dry propanol and air-dried; 0.24 g., specific rotation[8] +67.1 ± 1.6 (accepted[9] value for sucrose, +66.5).

The tobacco seed sucrose (0.2 g.) was acetylated using an equal weight of anhydrous sodium acetate and 2 ml. of acetic anhydride by heating to boiling on a hot-plate until the sucrose was completely dissolved. The reaction product was evaporated to dryness overnight in a warm air stream and the residue was extracted with benzene. The filtered extract was evaporated to dryness and taken up in hot butanol. The crystals which formed on cooling were removed

(1) Journal Paper No. J-2596 of the Iowa Agricultural Experiment Station, Ames, Iowa. Proj. 1116. Supported in part by a grant from the Corn Industries Research Foundation. Technical assistance was furnished by Miss Ana Pellecer.

(2) E. Wada and K. Yamazaki, *J. Agr. Chem. Soc. Japan*, **24**, 398 (1951).

(3) N. Wattiez and M. Hans, *Bull. Acad. roy. méd. Belg.*, **8**, 386 (1943).

(4) D. French, G. M. Wild, B. Young and W. J. James, THIS JOURNAL, **75**, 709 (1953).

(5) Purchased from T. W. Wood and Sons, Richmond, Virginia.

(6) A. Jeanes, C. S. Wise and R. J. Dimler, *Anal. Chem.*, **23**, 415 (1951); D. French, D. W. Knapp and J. H. Pazur, THIS JOURNAL, **72**, 5150 (1950).

(7) The procedure of R. L. Whistler and D. F. Durso was followed, except that no Celite was used in preparing the column; R. L. Whistler and D. F. Durso, THIS JOURNAL, **73**, 4189 (1951).

(8) Specific rotations reported are for dilute aqueous solutions (<4%) using sodium light.

(9) F. J. Bates, "Polarimetry, Saccharimetry and the Sugars," Circular C440 of the National Bureau of Standards, Washington, 1942.

TABLE I

COMPARISON OF PROPERTIES OF TOBACCO SEED TRISACCHARIDE WITH THOSE OF PLANTEOSE

Property	Planteose	Tobacco seed trisaccharide
Products of complete acid hydrolysis	D-Glucose, D-fructose, D-galactose[a]	Same[b]
Products of partial acid hydrolysis	D-Glucose and a ketose disaccharide, planteobiose[c]	D-Glucose and a ketose disaccharide[b]
Action of yeast invertase	None[a]	None
Action of α-galactosidase	Sucrose and D-galactose	Same[b]
Specific optical rotation	+125.5,[a] + 130[c]	+127.2
M.p. of the acetate	139, 135[c]	137° (mixed m.p. 137°)
Paper chromatographic $R_f/(1 - R_f)$ values[d] for the trisaccharide	0.20 ± 0.02	0.19 ± 0.01
Same for the ketose disaccharide	0.45 ± 0.03	0.47 ± 0.00
X-Ray powder pattern of the crystalline trisaccharide	Characteristic	Identical with that of planteose

[a] Ref. 3. [b] Ref. 2. [c] Ref. 4. [d] D. French and G. M. Wild, THIS JOURNAL, **75**, 2612 (1953). The data given refer to the solvent butanol–pyridine–water 6:4:3 parts by volume.

TABLE II

CHARCOAL COLUMN SEPARATION OF TOBACCO SUGARS

% Ethanol in eluate	Obsd. rotation (4 dm. tube)	Chromatographic composition
0 (put-on water)	0.025	(Discarded)
2	.072	Sucrose
4	.069	Sucrose–trisaccharide 4:1
6	.183	Sucrose–trisaccharide 1:10, trace of ketose disaccharide
8	.065	Same as 6
10	.017	Same as 6 plus a trace of fructose-containing tetrasaccharide
40 (500 ml.)	.009	Small amount of fructose, sucrose and trisaccharide

by filtration, washed with butanol and air-dried; m.p.[10] 88°, mixed m.p. with authentic sucrose octaacetate 88°, sucrose octaacetate, 89°.

Crystallization of Tobacco Seed Trisaccharide.—The charcoal column 6–10% ethanol eluates were dissolved in a small volume of water, combined, filtered and evaporated to a heavy sirup. The sirup was treated with 85% ethanol and seeded very lightly with planteose. Crystallization of the sirup took place over a two-day period. The pasty mixture of crystals and sirup was diluted with 80% methanol and filtered. The crystals were washed first with dilute methanol, then with propanol, and air-dried; 0.18 g., specific rotation +119. To purify the crystals were dissolved in 5 ml. of boiling 85% ethanol, the solution was filtered and the filtrate was seeded with the crude crystals. On standing overnight at 0°, a small sharply crystalline deposit formed, which was removed by filtration, washed with 85% ethanol and propanol, and air-dried; 0.03 g., specific rotation +127.2 ± 0.4 (literature values for planteose, +125.5,[3] +130[4]).

X-Ray Diffraction Data for Tobacco Seed Trisaccharide.—The X-ray diffraction powder patterns of tobacco seed trisaccharide and planteose were identical: 5.74,[11] 70[12]; 5.16, 100; 4.54, 10; 4.31, 35 (double); 4.17, 5; 3.97, 20 (double); 3.78, 10; 3.62, 55; 3.46, 25; 3.34, 10; 3.27, 10; 3.04, 10; 2.94, 10; 2.35, 20; 2.24, 20.

Tobacco Seed Trisaccharide Acetate.—The solution of crystalline tobacco seed trisaccharide used for determination of the optical rotation was evaporated to dryness and acetylated as described for the sucrose fraction. The crystals which formed were removed by filtration, washed with butanol, and air-dried; m.p. 137°, mixed m.p. with authentic planteose acetate 137°, planteose acetate 139°.

Action of Yeast on Tobacco Seed Sugars.—The 4% ethanol eluate from the charcoal column was evaporated to 5% solids concentration, acidified to *p*H 4 with acetic acid and treated with an amount of active dried bakers' yeast equal to the total weight of sugars present. After 48 hours at room temperature, the digest was filtered through fritted glass to remove yeast cells. Paper chromatography showed that the sucrose had been entirely removed, although trisaccharide still remained. Large scale paper chromatography of the non-fermented residue gave a sirup which crystallized from 85% ethanol after seeding with a minute trace of planteose crystals.

Paper Chromatographic Data.—Part of the 4% eluate after fermentation was hydrolyzed by heating in 3 *N* hydrochloric acid at 70° for 3 minutes. The solution was cooled and neutralized with pyridine. The tobacco trisaccharide and its derived ketose were then compared with planteose and planteobiose on single and double ascent paper chromatograms. Averages and mean deviations of the $R_f/(1 - R_f)$ values are recorded in Table I. Each value given is the average of about 6 separate determinations.

AMES, IOWA

(10) Melting points reported were obtained using a hot stage and are uncorrected.

(11) *d* value in Å.

(12) Relative intensity on basis of 100 for strongest line.